

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

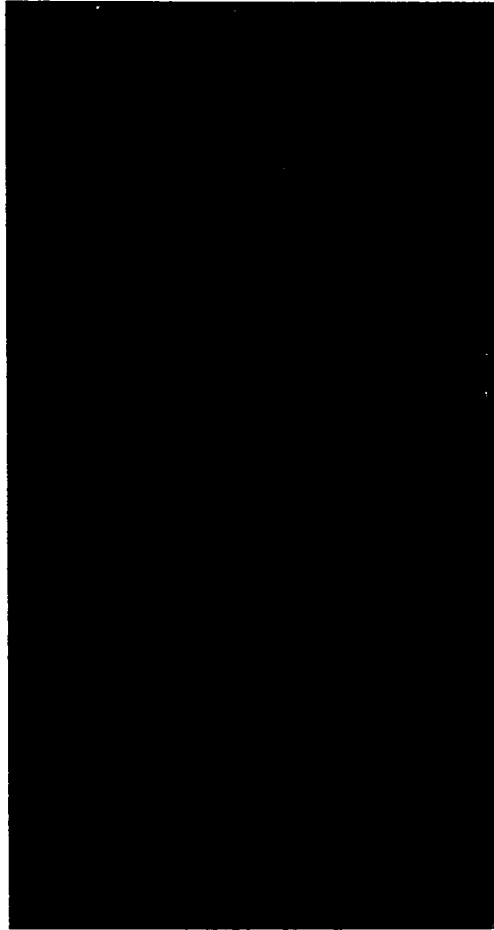
**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**



GFP-LCA (wt)

**FIG. 1**

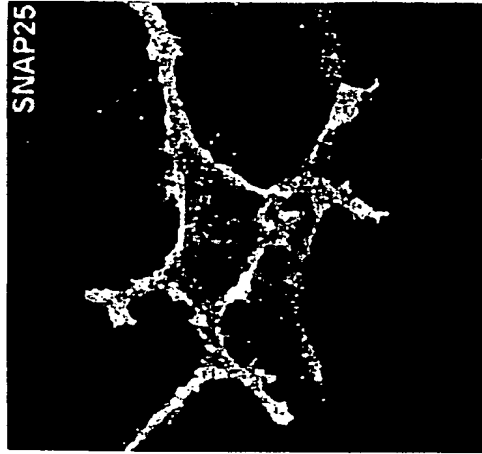


**GFP-TLCA**

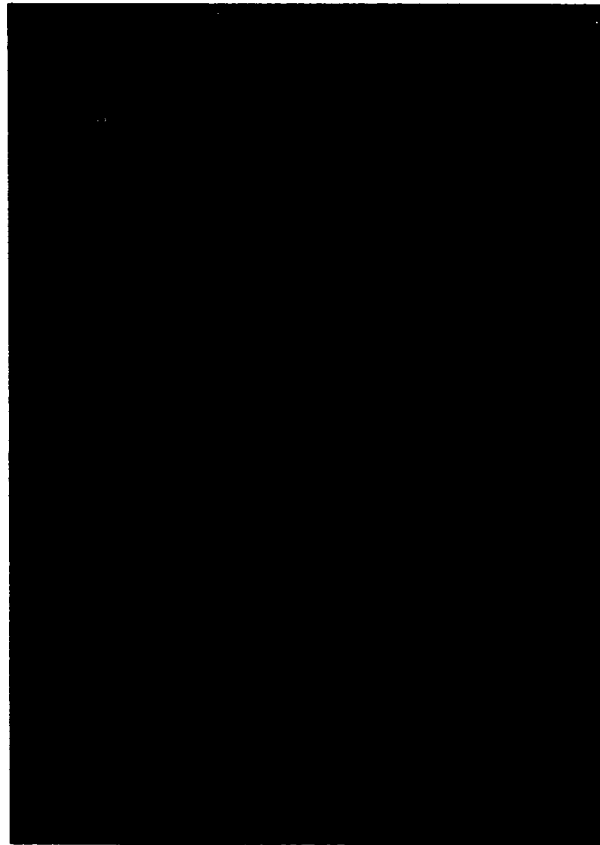
**FIG. 2**

1 PFVNKQENYK DPVNGVDIAY IKIPNVGQMQ PVKAFKIHNK IWVIPERDTF  
 51 TNPEEGDLNP PPEAKQVPVS YDSTYLSTD NEKDNYLKGV TKLFEIYST  
 101 DLGRMLLTSI VRGIPFWGGS TIDTELKVID TNCINVIQPD GSYRSEELNL  
 151 VIIGPSADII QFECKSFGHE VLNLTRNGY STQYIRFSPD FTFGFEESLE  
 201 VDTNPLLGAG KFATDPAVTL AHELIHAGHR LYGIAINPNR VFKNVTNAYY  
 251 EMSGLEVSFE ELRTFGGHA KFIDSLQENE FRLYY<sup>\*</sup>YNKFK DIASTLNKAK  
 301 SIVGTTASLQ YMKNVFKEKY LLSEDTSGKF SVDKLKFDKL YKMLTEIYTE  
 351 DNFVKFFKVL NRKTY<sup>\*</sup>LNFDK AVFKINIVPK VNYTI<sup>\*</sup>YDGFN LRNTNLAANF  
 401 NGQNT EINNM NFKLKNFTG LFEFYKLLCV RGIITSK

FIG. 3



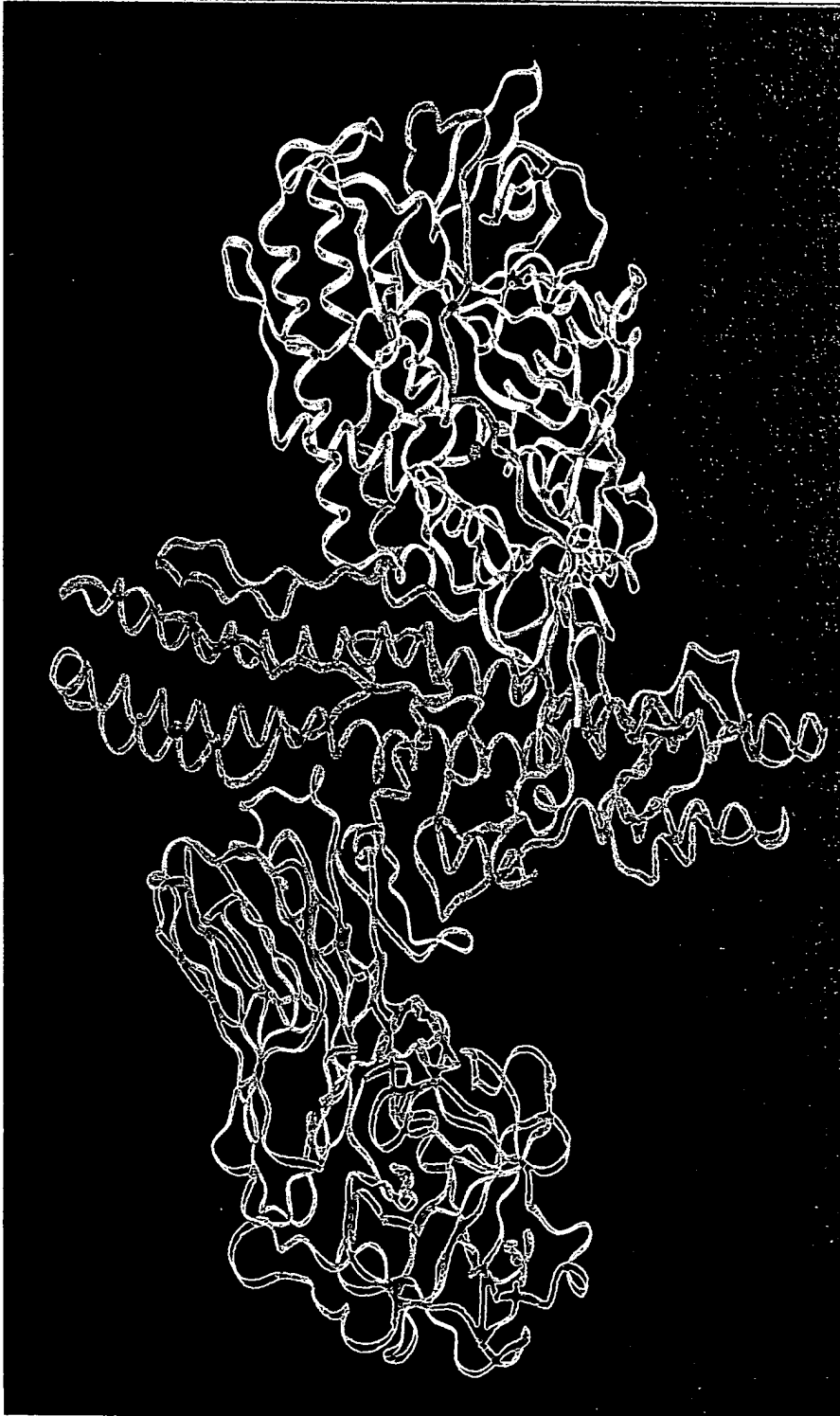
**FIG. 5**



**FIG. 4**

Botulinum Toxin

Type A X-Ray Structure

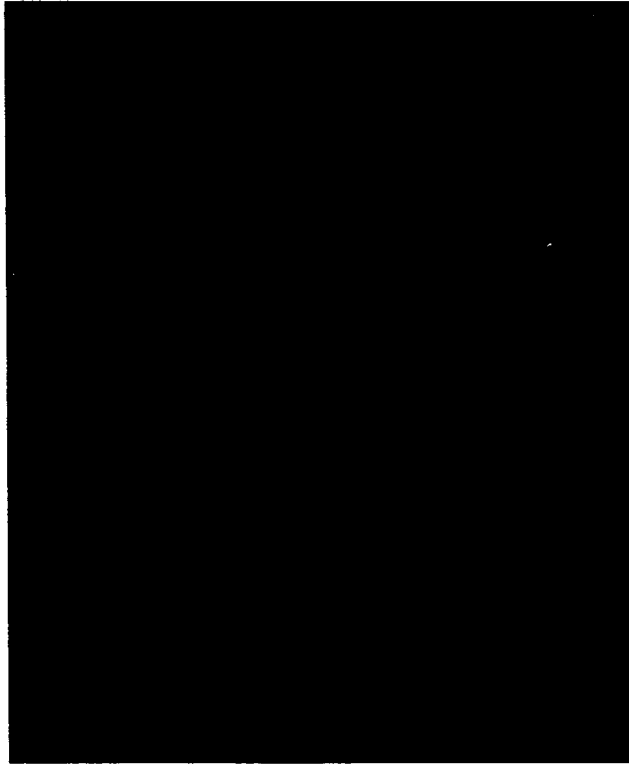


Yellow = protease domain

Green = binding domain

Red = translocational domain

FIG. 6

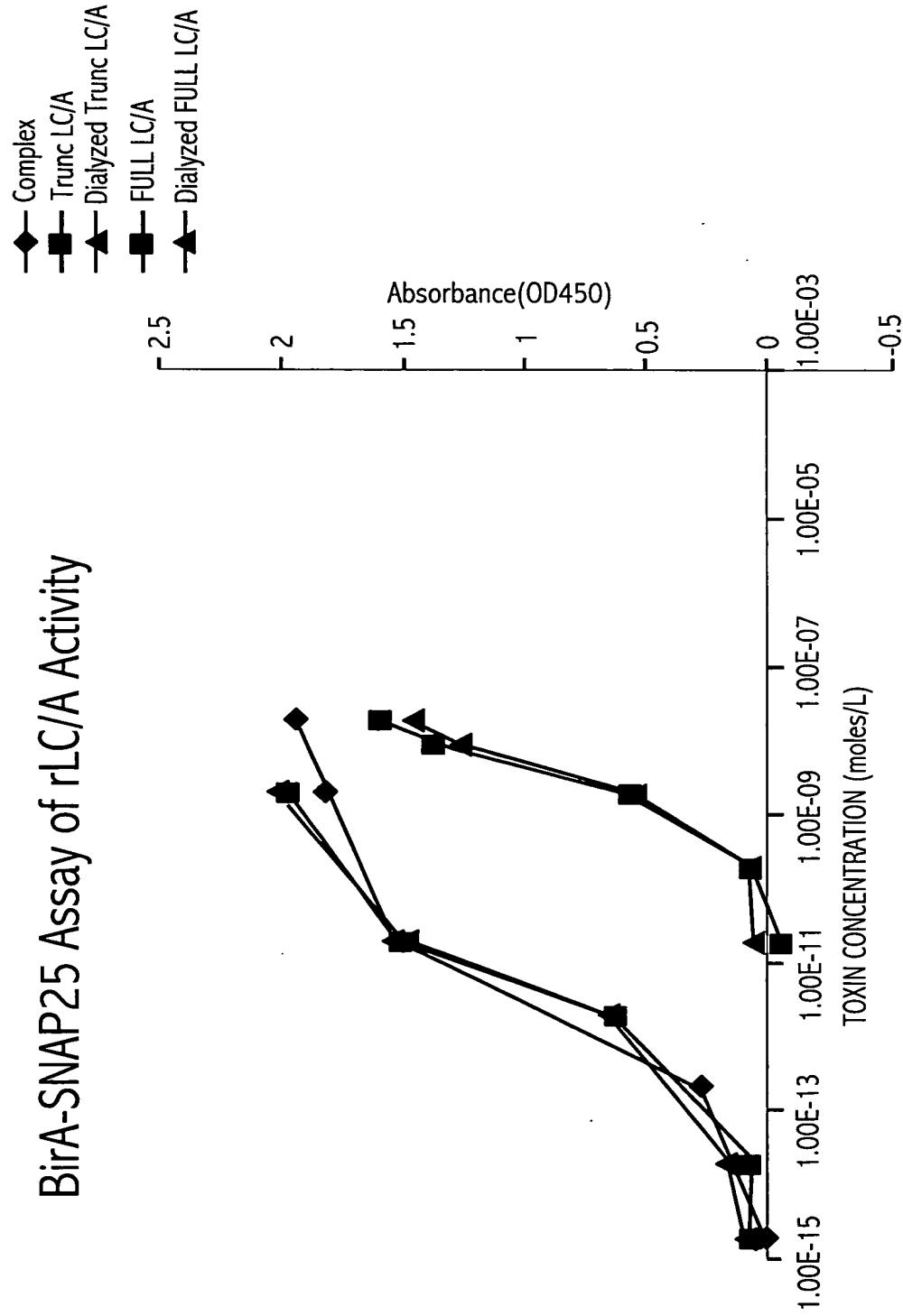


GFP-LCB (wt)

**FIG. 7**

[illegible]**FIG. 8**



**FIG. 9**

# Comparison of LC/A constructs expressed from E. coli for in vitro analysis

|               | 6xHis                 | S-tag                    |                 |
|---------------|-----------------------|--------------------------|-----------------|
| Wildtype LC/A |                       |                          |                 |
| trun LC/A     | MHHHHHSSGLVPRGSGMKETA | AKFERQHMDSPDLGTDDDDKAM   | .....YKDPV...   |
| N-His LC/A    | MHHHHHSSGLVPRGSGMKETA | AKFERQHMDSPDLGTDDDDKAMGS | FVNKQFNYKDPV... |
| Wildtype LC/A | ...NFTKLKNFTGL        | FEFYKLLCVRGI             | ITSK            |
| trun LC/A     | ...NFTKL              | .....TRAHHHHH            |                 |
| N-His LC/A    | ...NFTKLKNFTGL        | FEFYKLLCVRGI             | ITSK            |
|               |                       | 6xHis                    |                 |

9/36

Truncated LC construct published

- Kadkhodayan, S. et al. Prot. Exp. Purif. 2000, 19, 125-130
- Crystal structure reported at IBRCC in Oct. 2000

FIG. 10



FIG. 11



FIG. 12

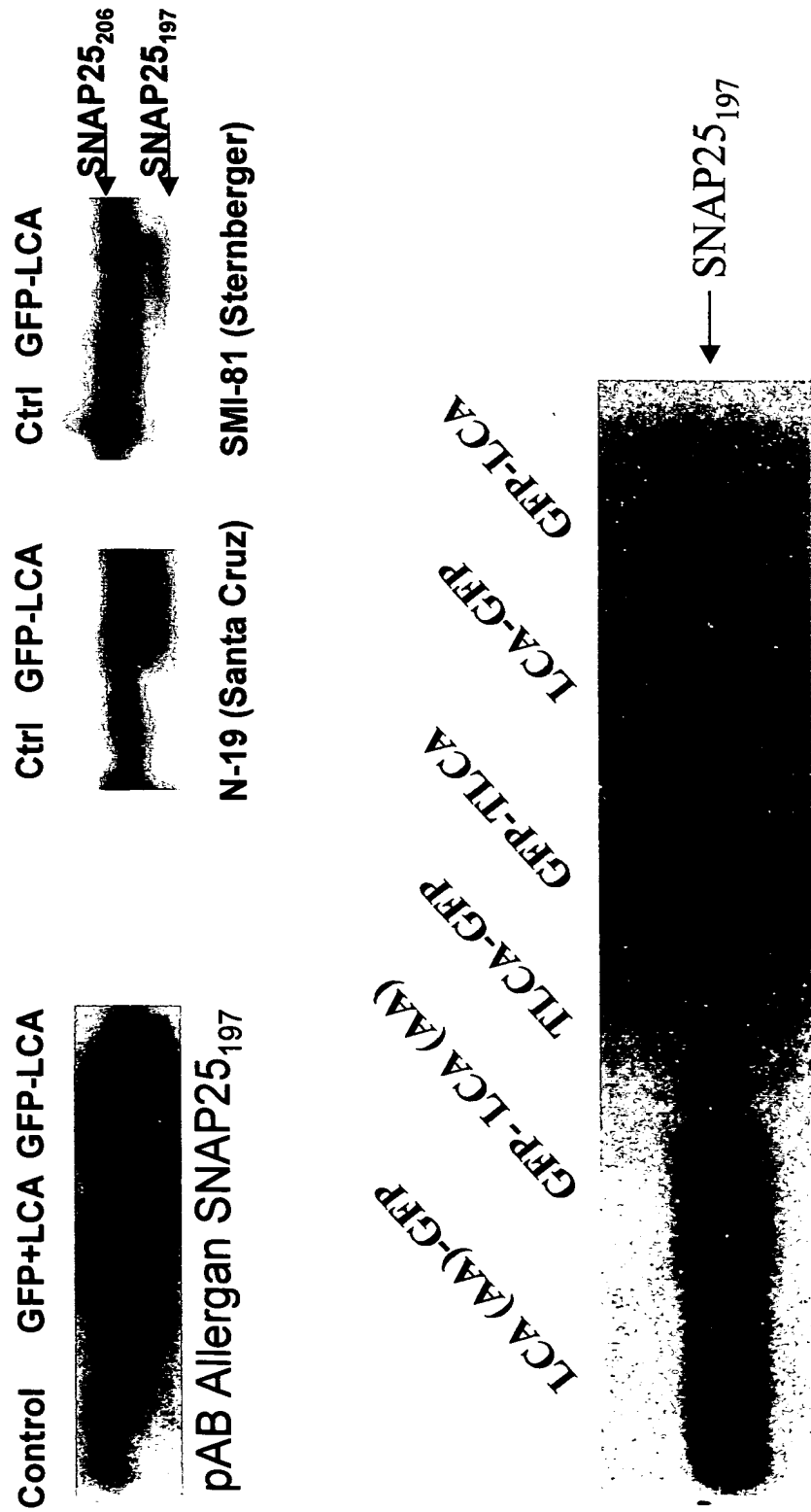


FIG. 13

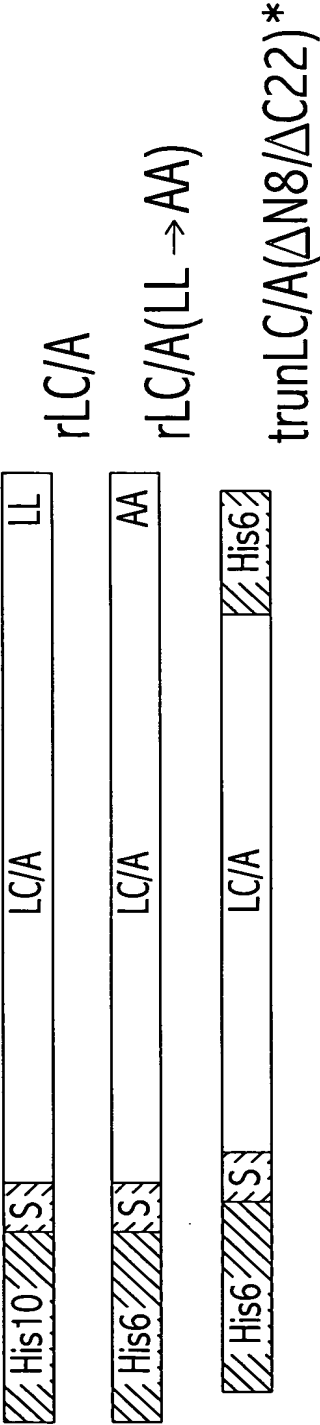


FIG. 14

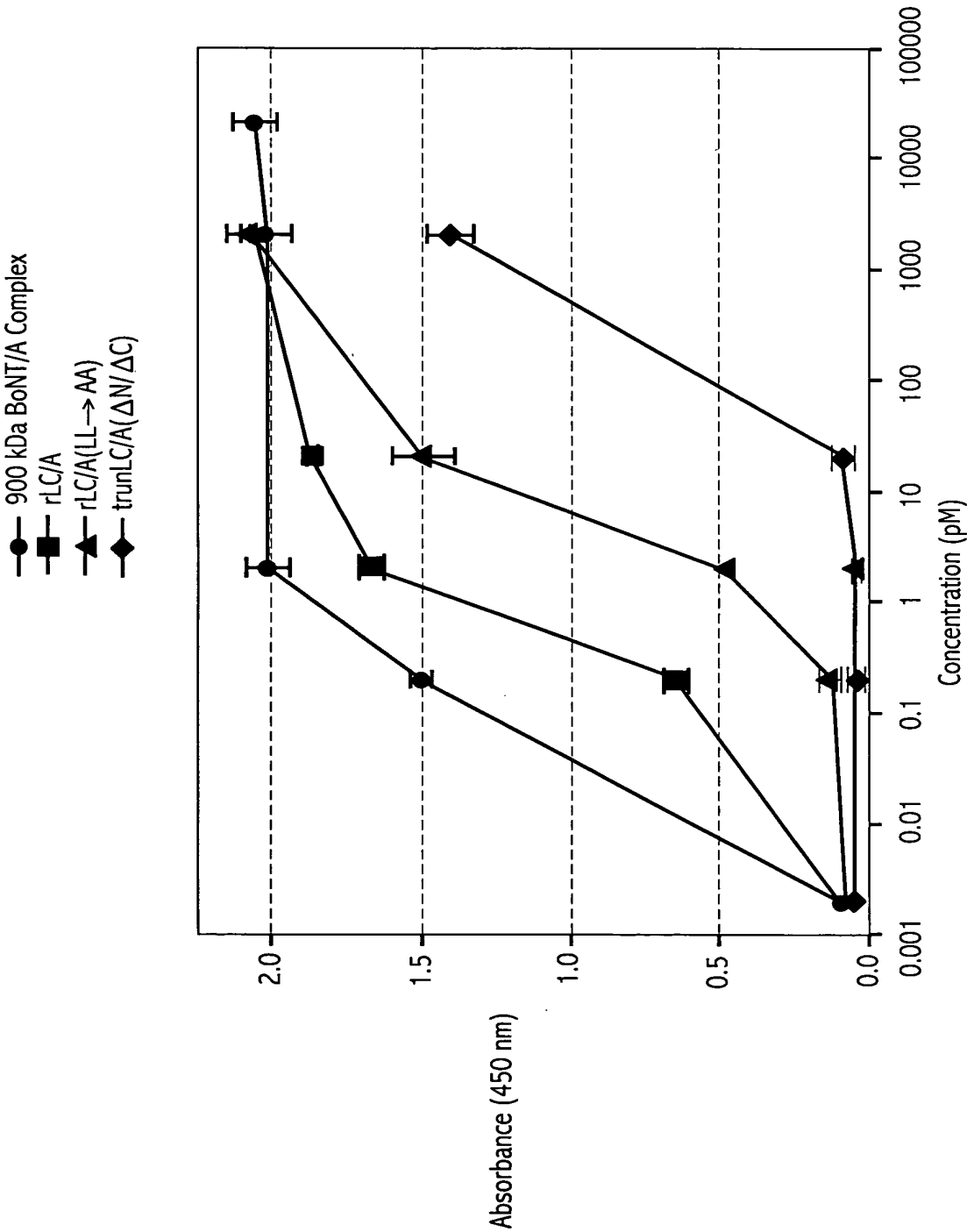
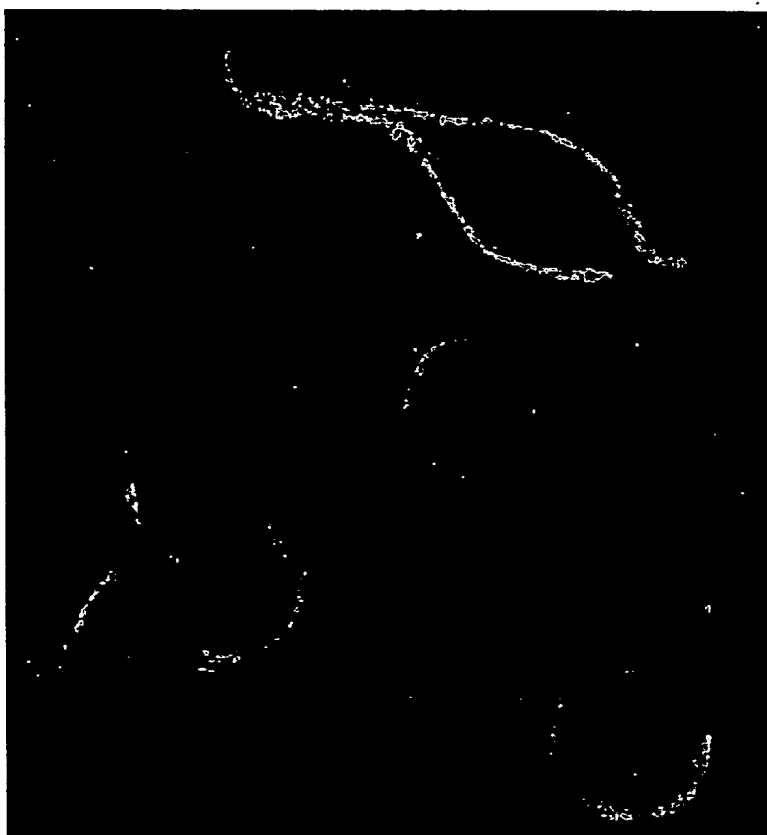
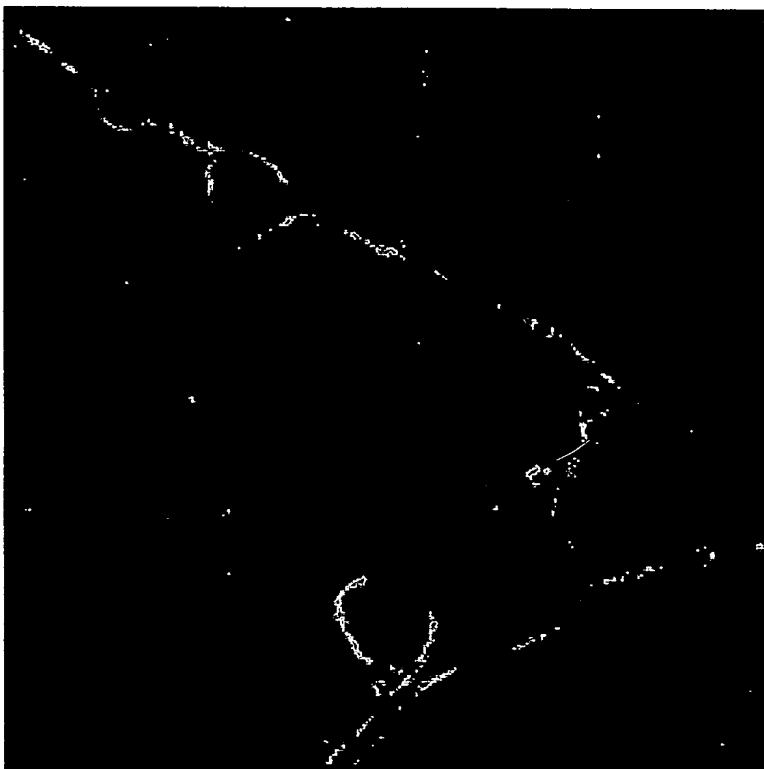
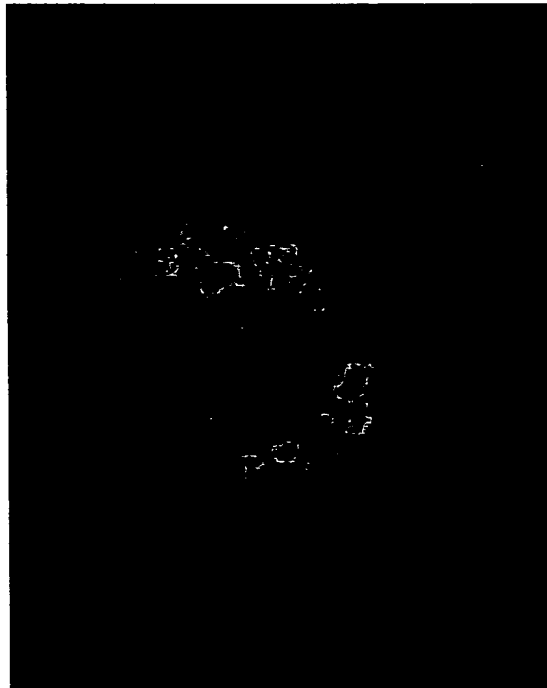
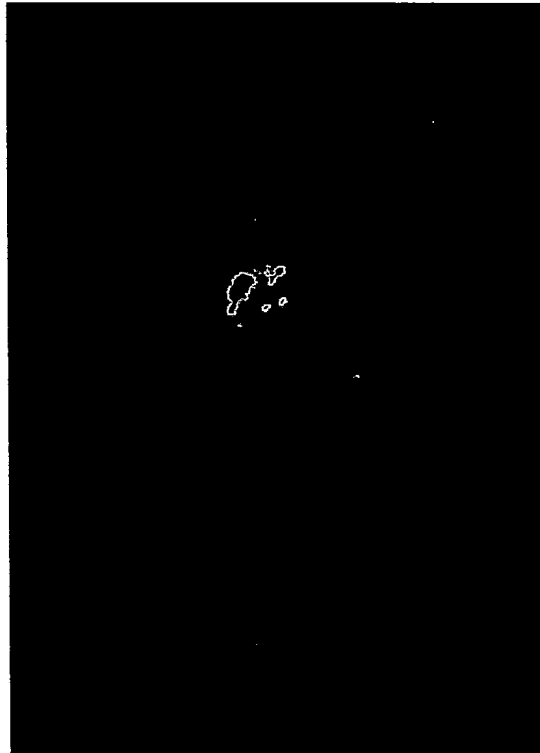


FIG. 15



**FIG. 16**



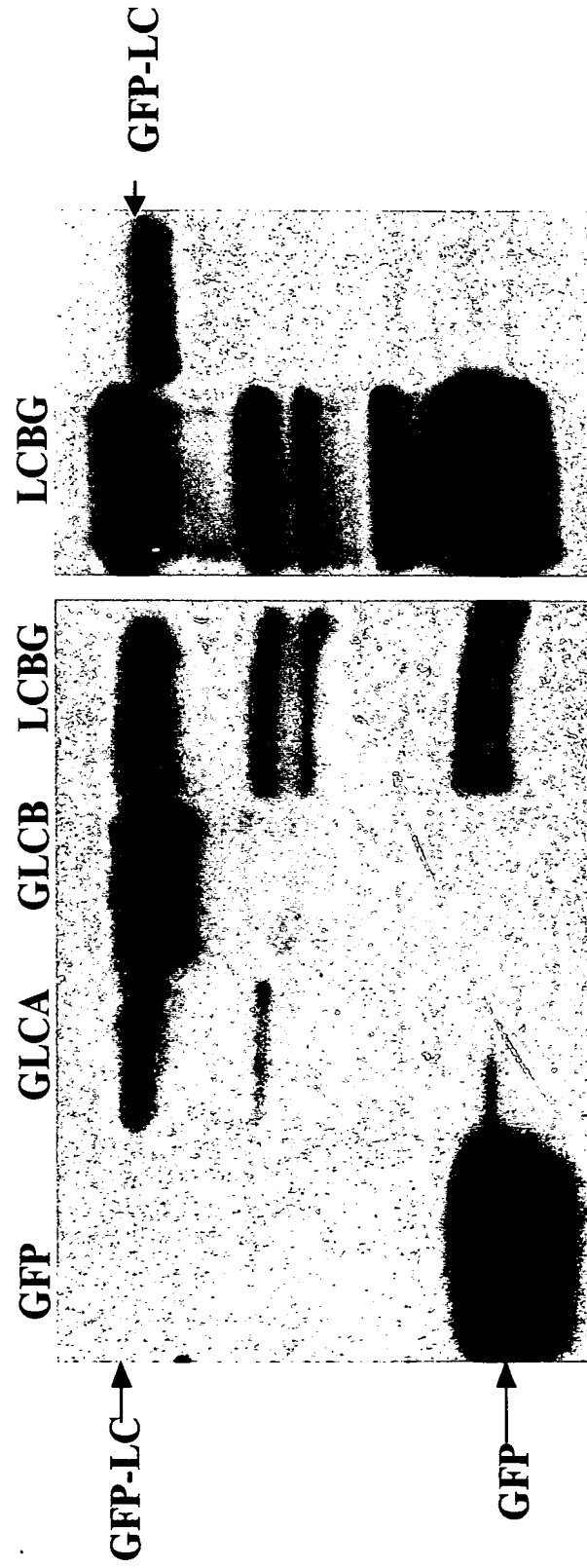


**FIG. 17**



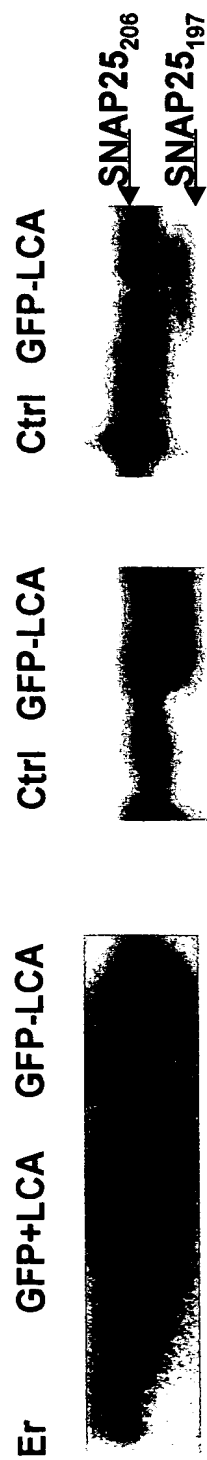
**FIG. 18**





IP: GFP (3E2) // WB:GFP (PolyAb)

**FIG. 19**

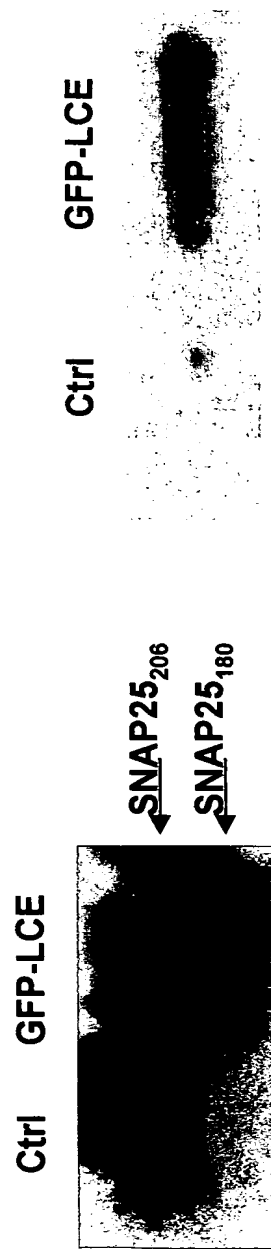


Ab to SNAP25<sub>197</sub>

N-19 (Santa Cruz) SMI-81 (Sternberger)

Ab to N-terminus SNAP25<sub>206</sub>

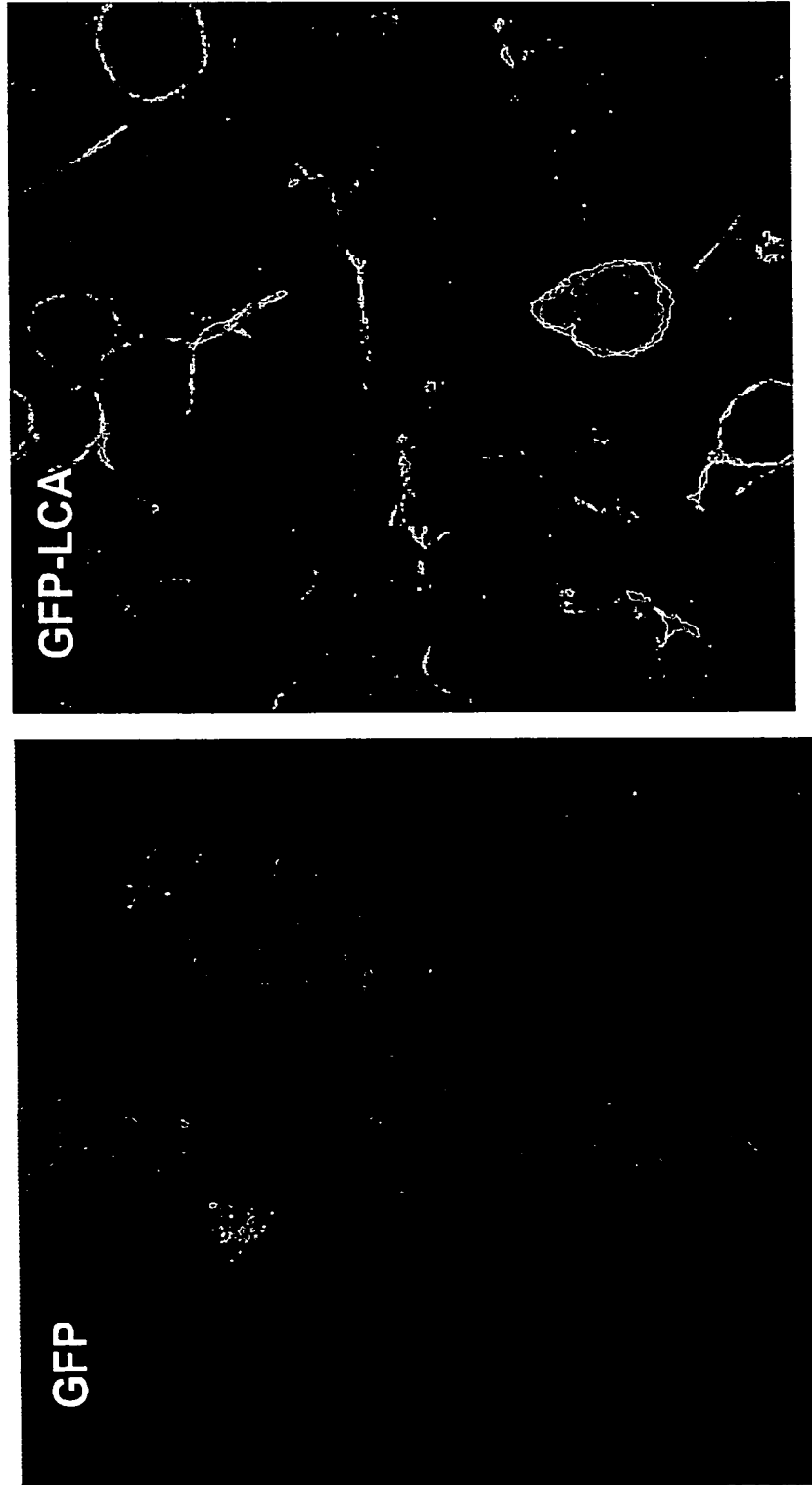
SNAP25<sub>206</sub>  
SNAP25<sub>197</sub>



WB: N19 antibody  
Ab to N-terminus SNAP25<sub>206</sub>

Ab to SNAP25<sub>180</sub>

FIG. 20



**FIG. 21**

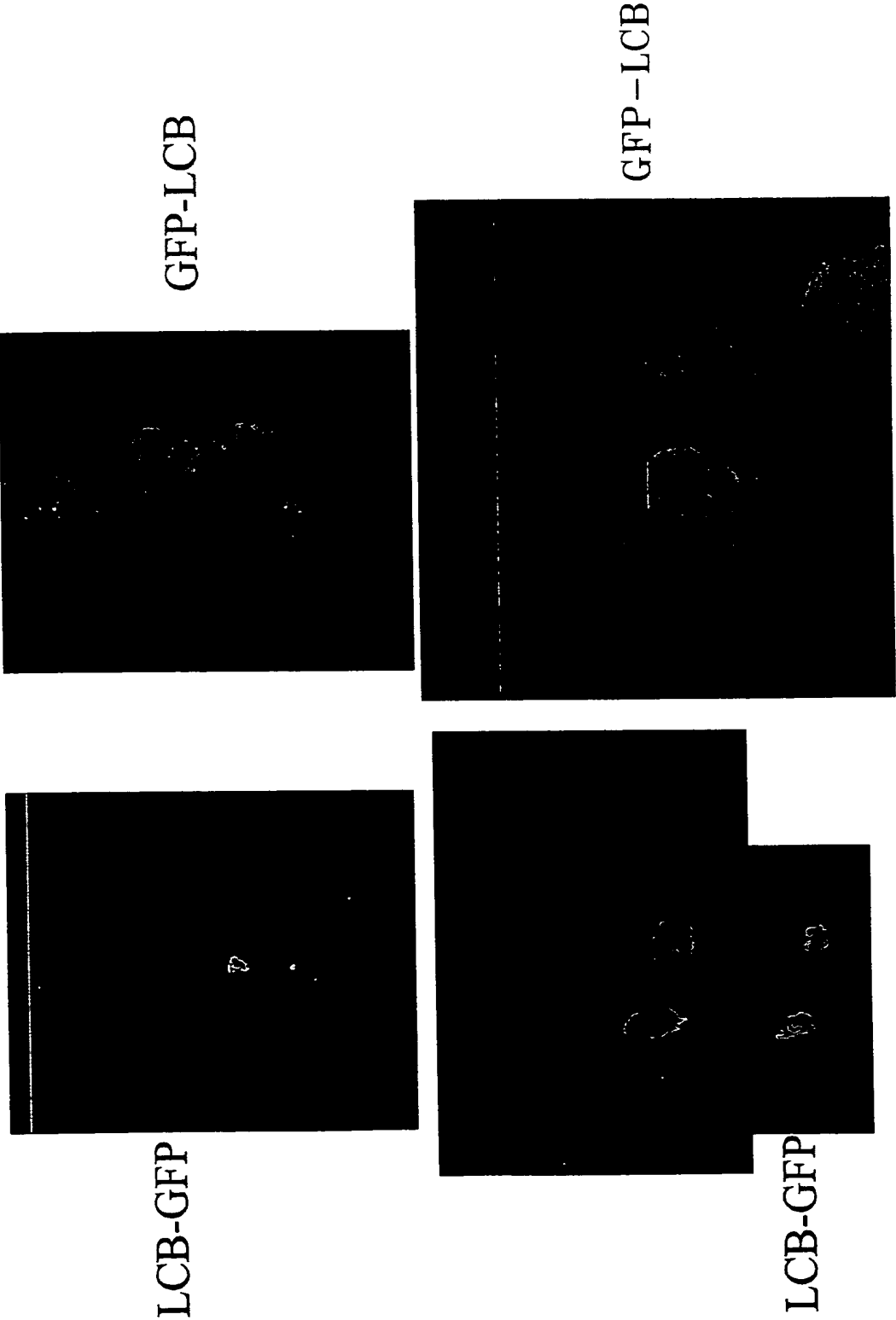


FIG. 22

Confocal

22/36

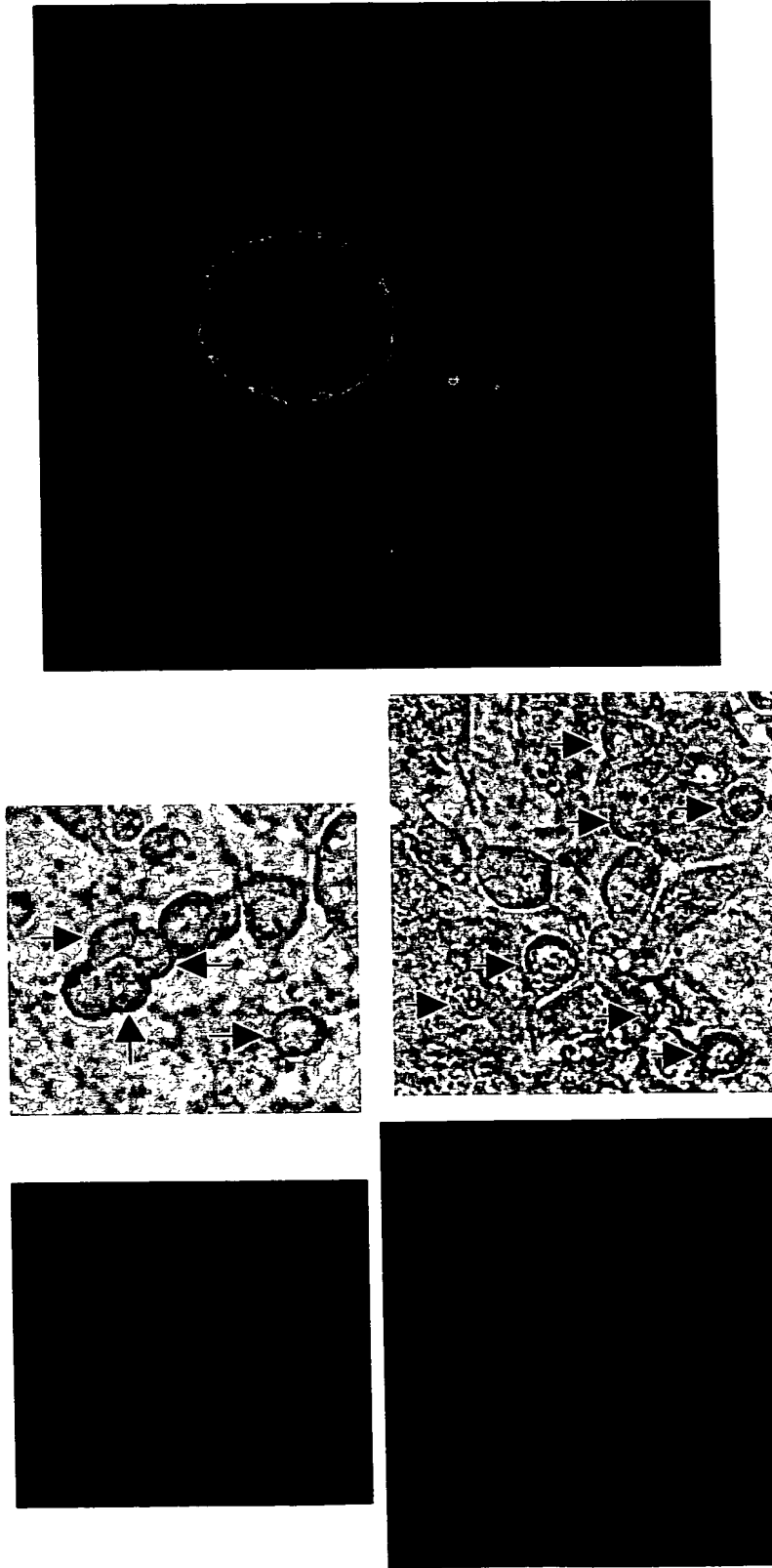
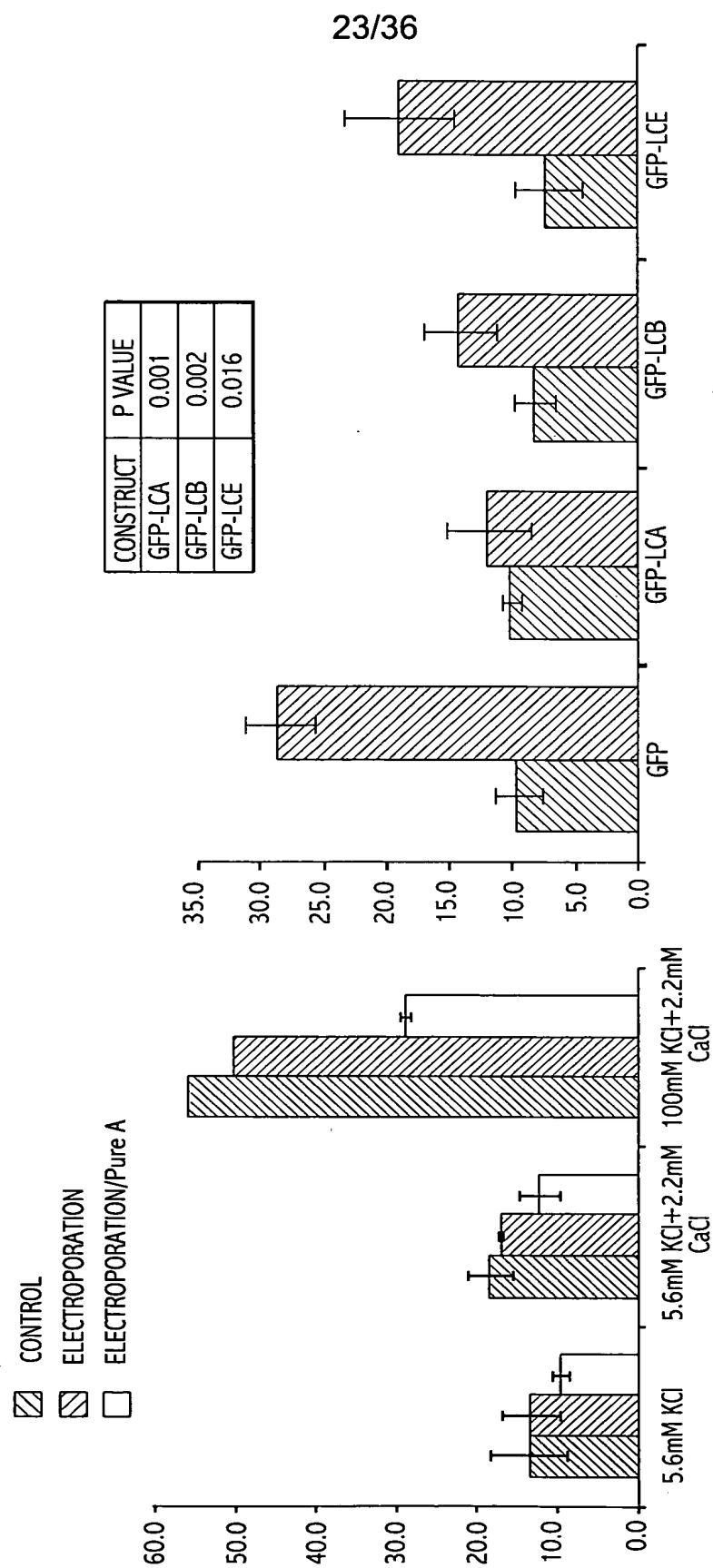
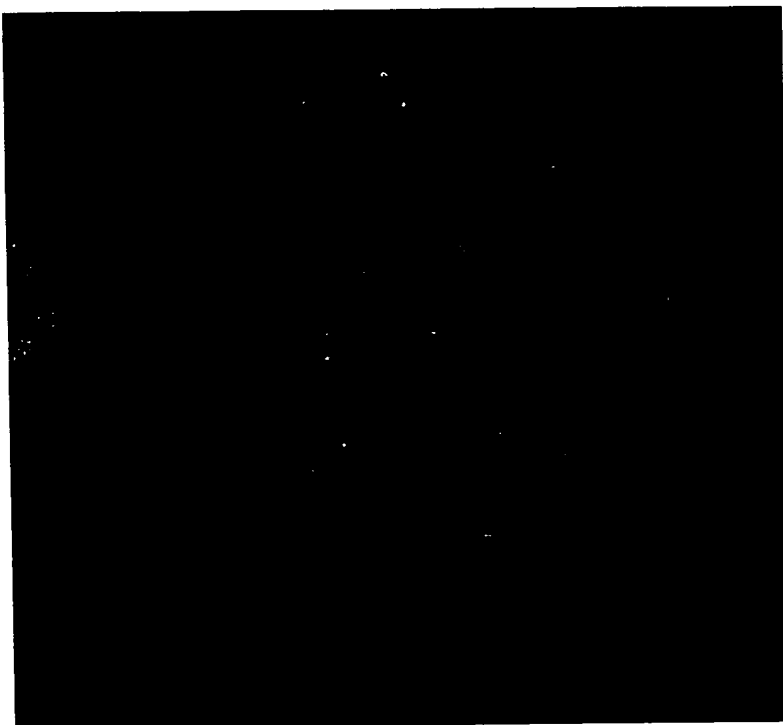


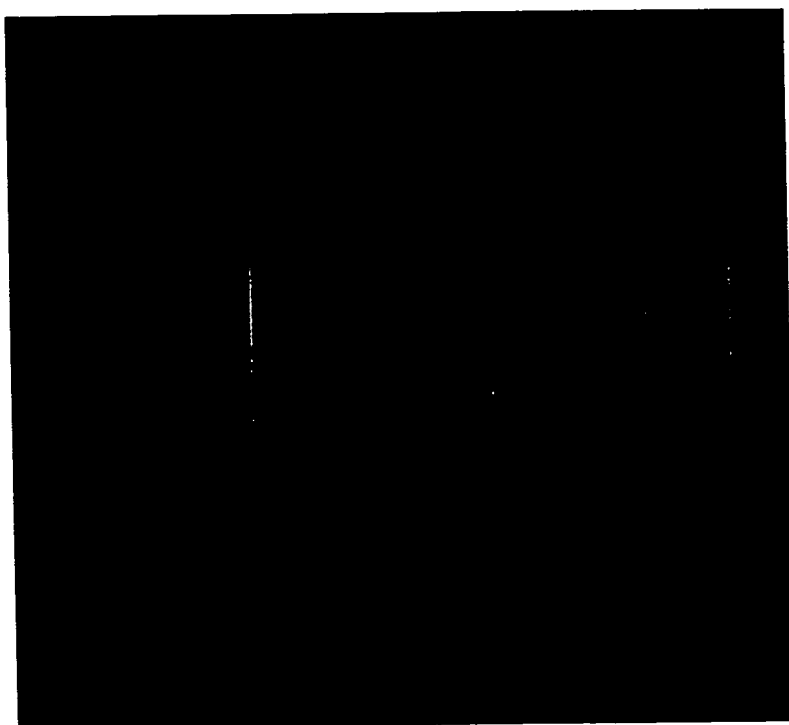
FIG. 23







HEK293T



HeLA

**FIG. 25**

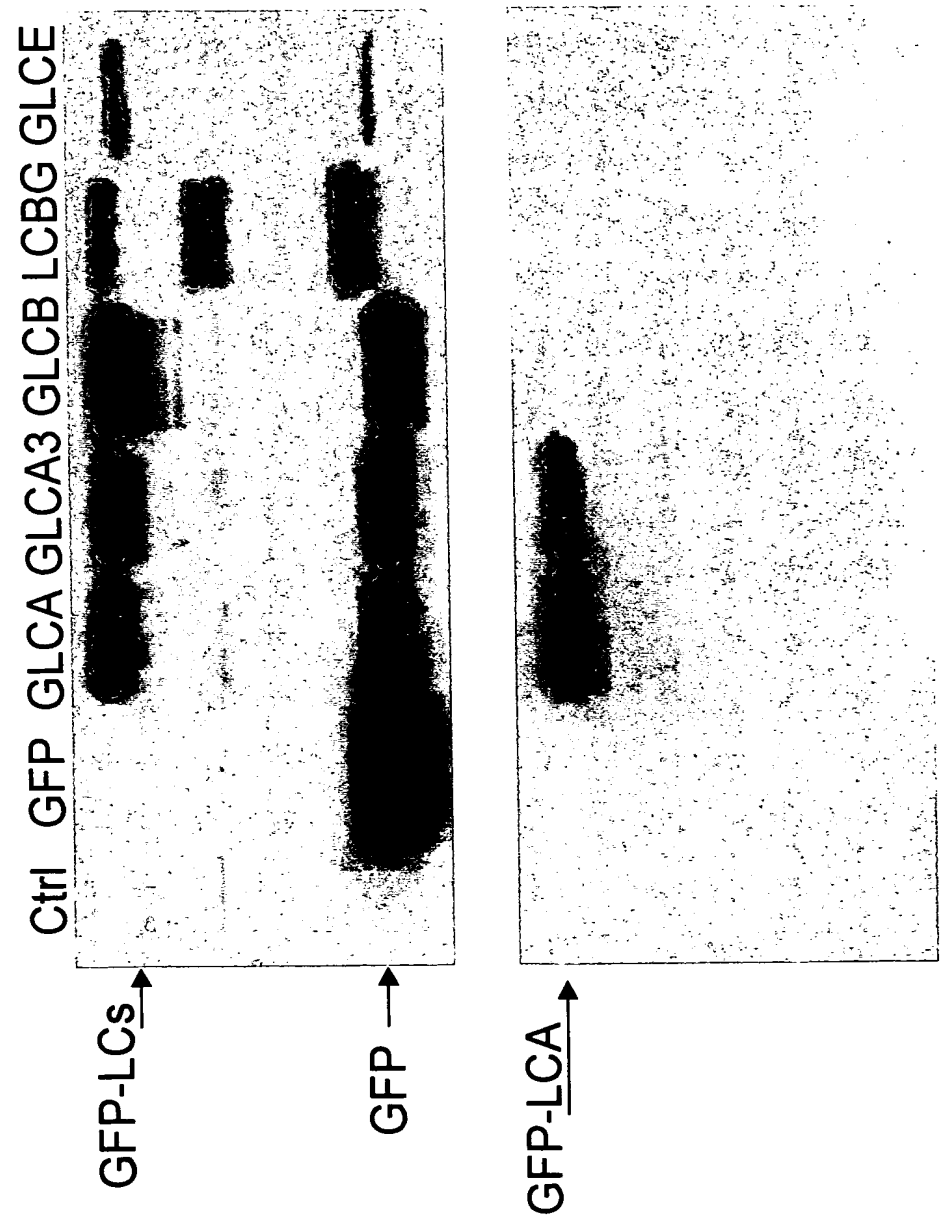
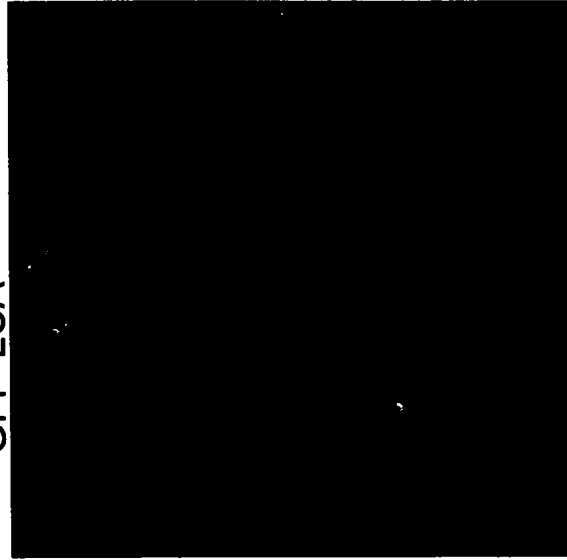
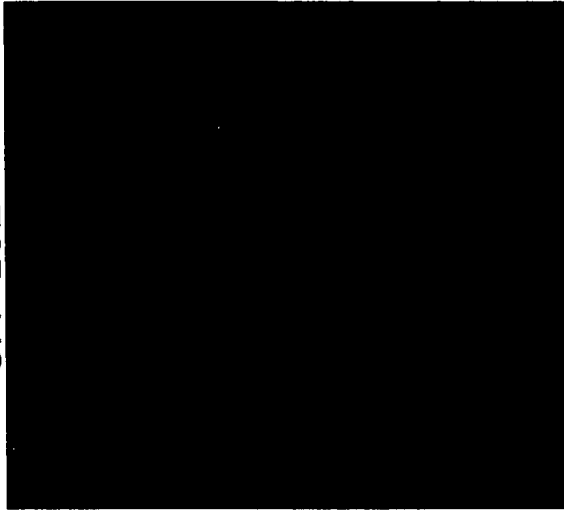


FIG. 26

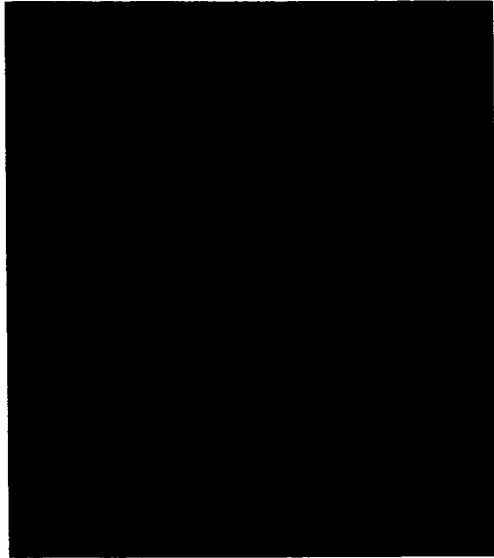
GFP-LCA



GFP-LCE



GFP-LCB



LCB-GFP

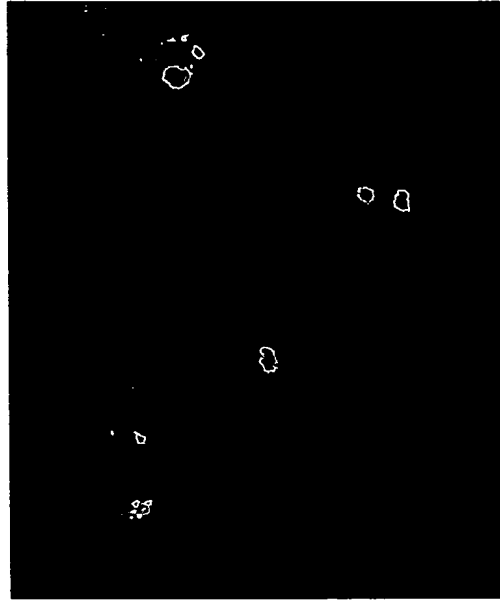


FIG. 27

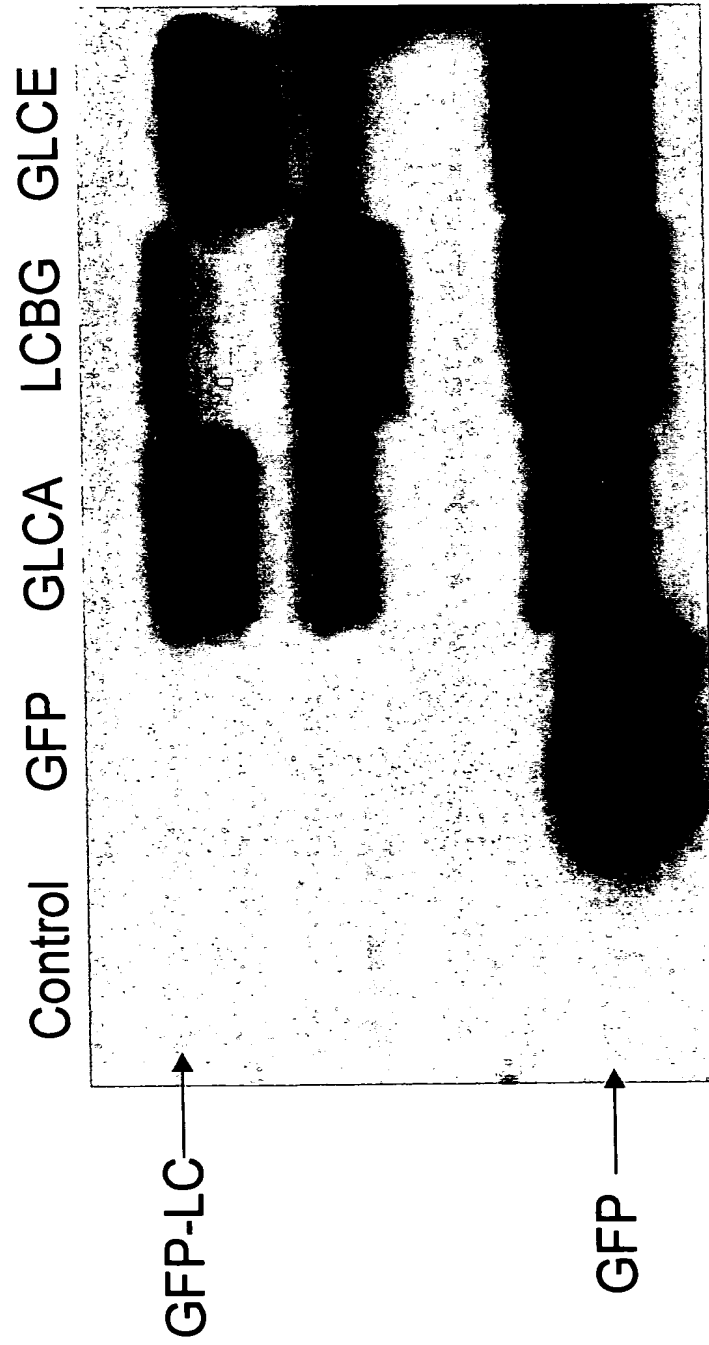
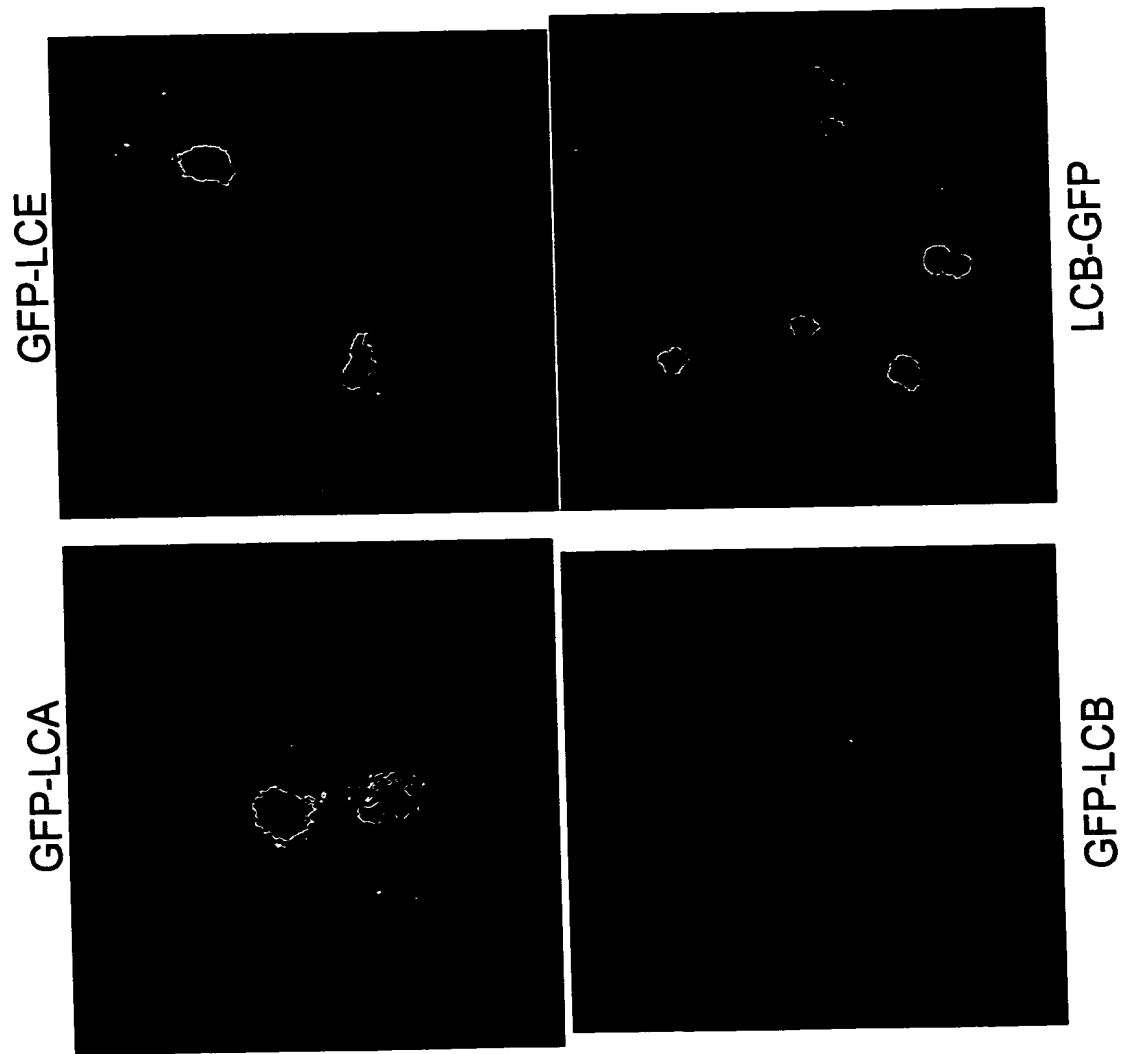


FIG. 28



**FIG. 29**

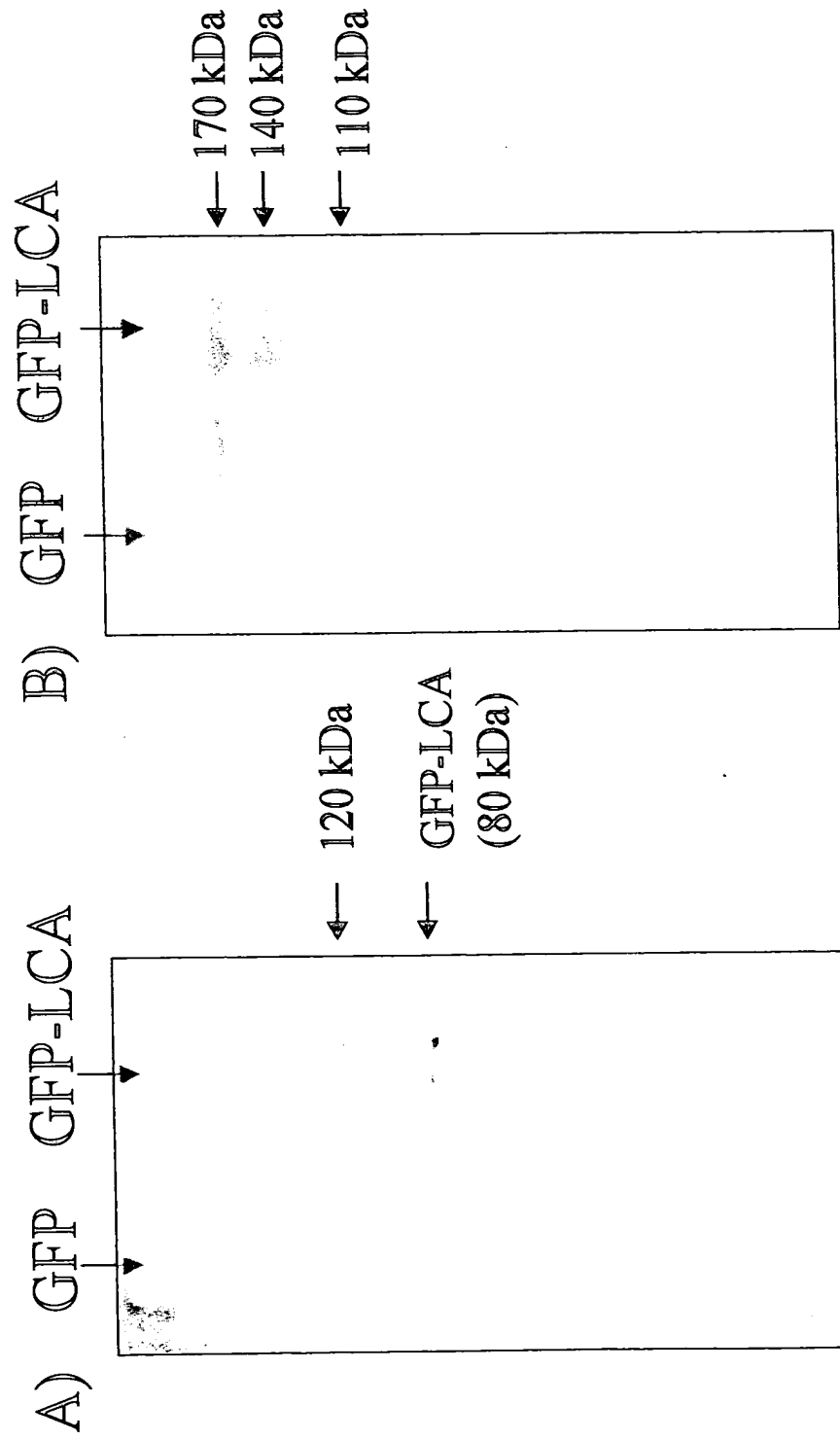
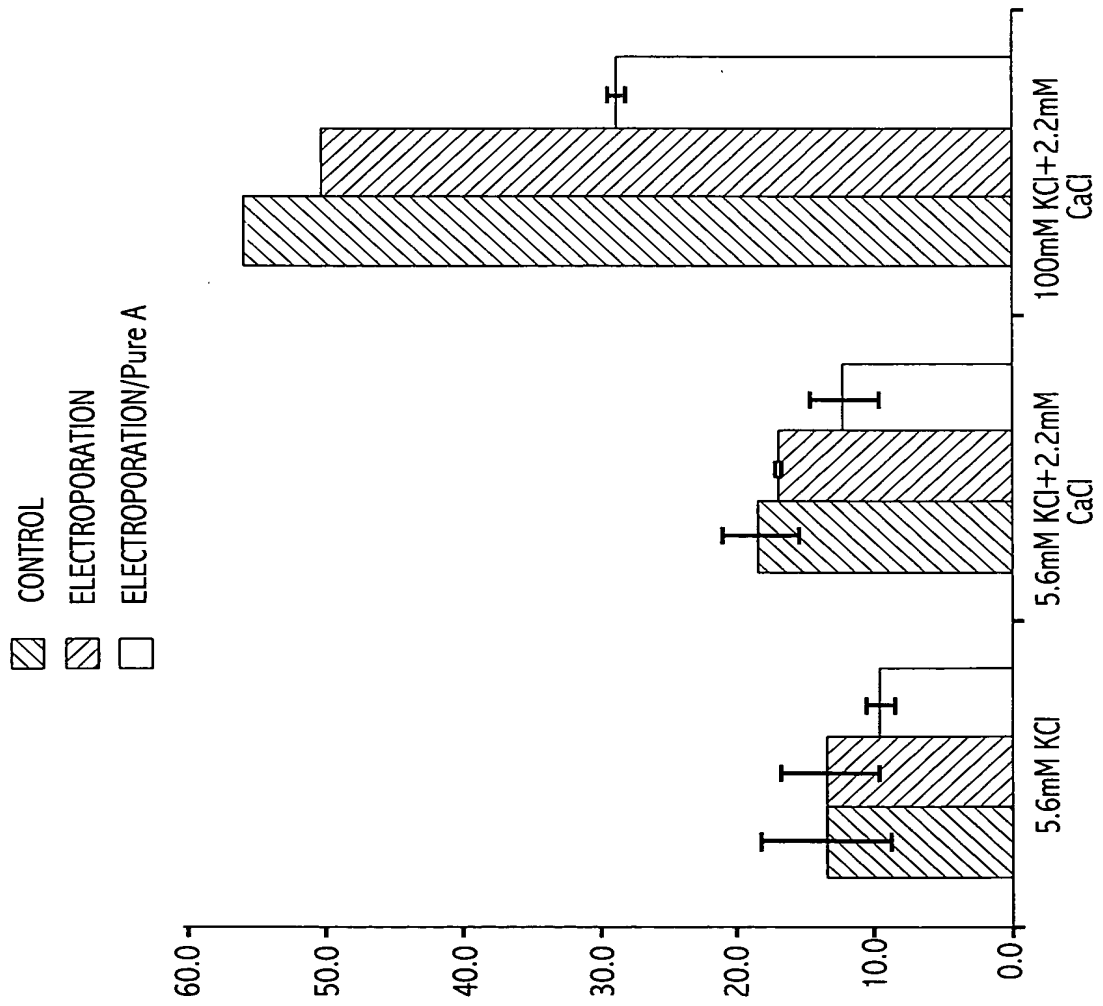


FIG. 30



**FIG. 31**

**FIG. 32**



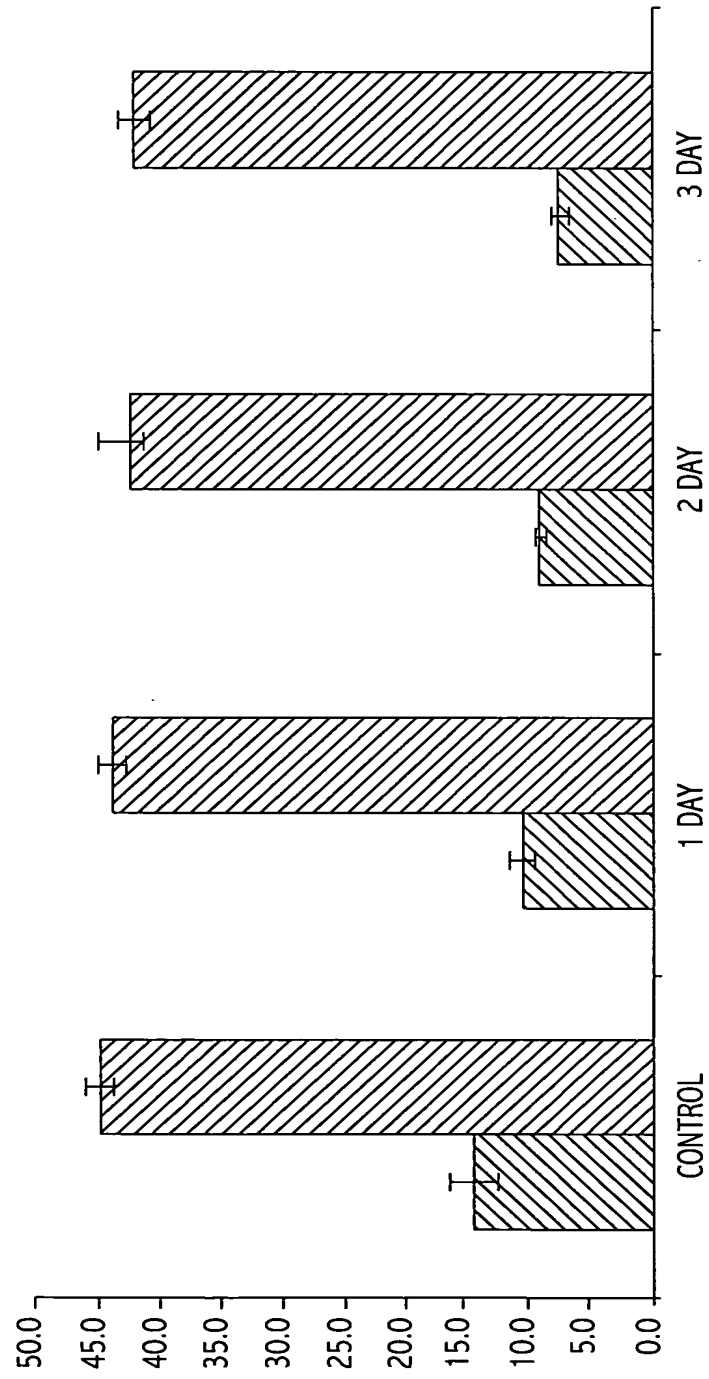
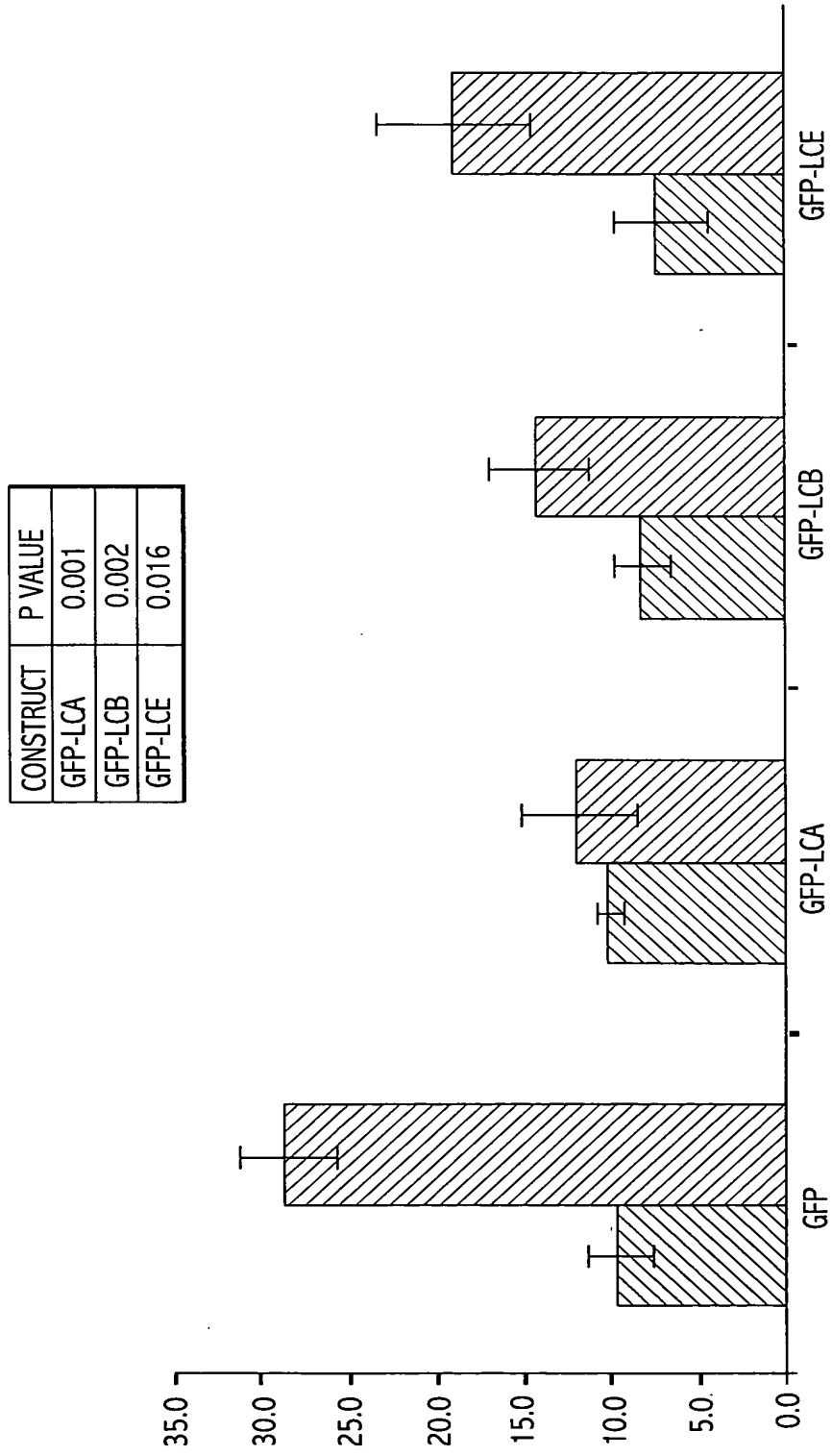
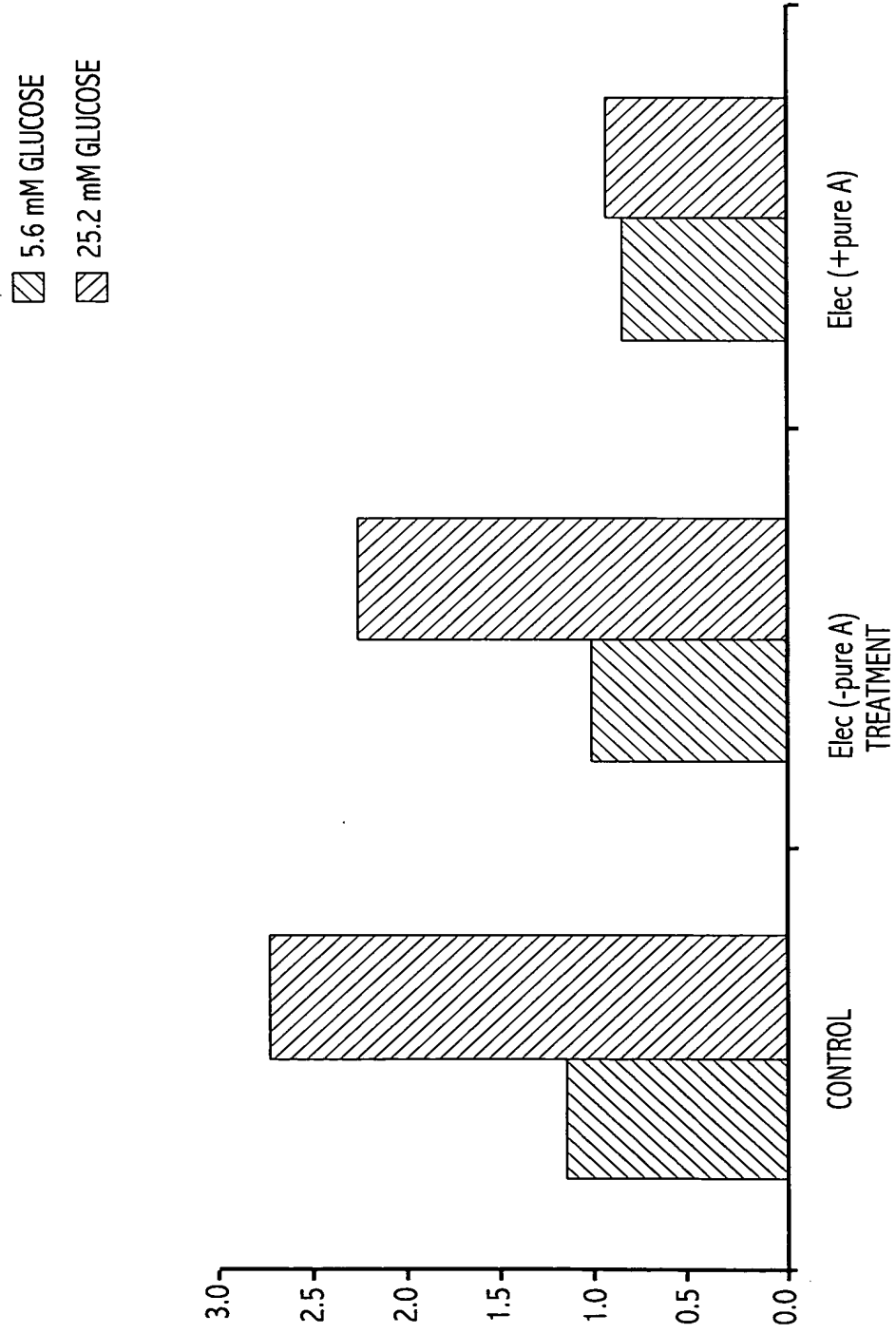


FIG. 33

**FIG. 34**

**FIG. 35**

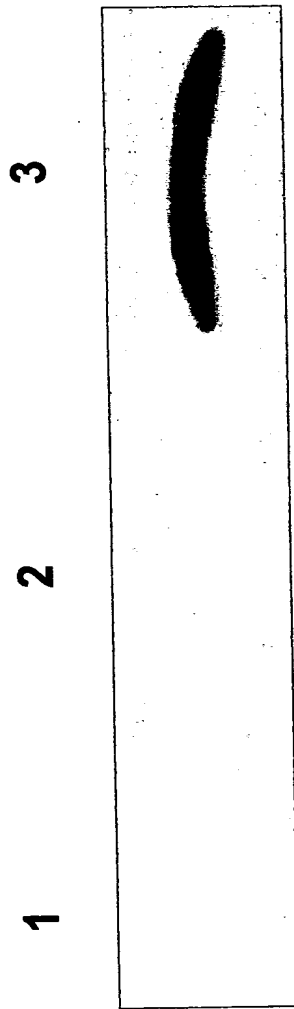
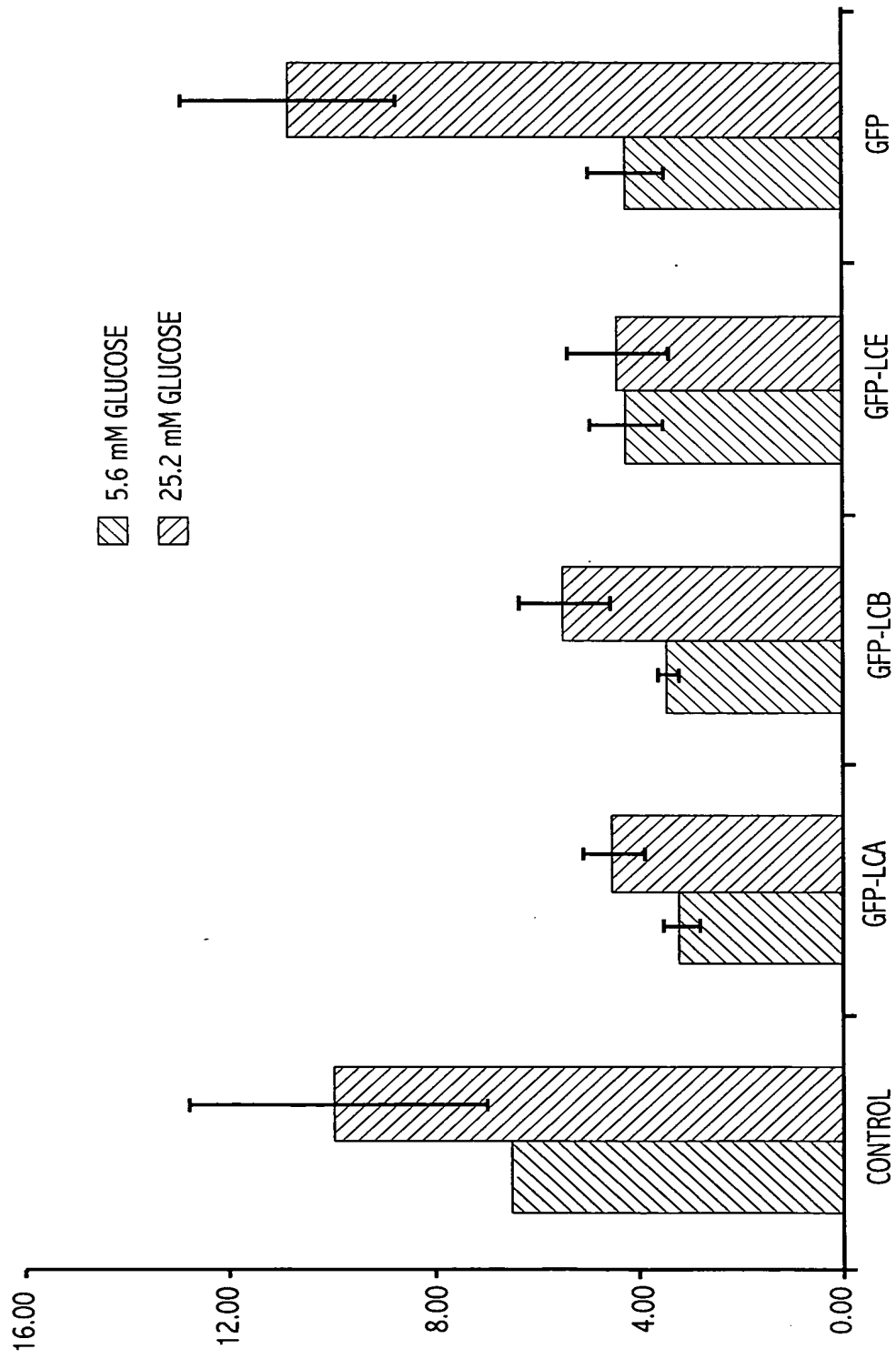


FIG. 36

**FIG. 37**